ELSEVIER

Biochimica et Biophysica Acta 1230 (1995) 202–206

BBA Biochimica et Biophysica Acta

Short Sequence-Paper

Cyanidium caldarium genes encoding subunits A and B of V-ATPase

Karl Ziegler [a], Günter Hauska [b], Nathan Nelson [c,*]

[a] *Fachbereich Biologie, Humboldt Universitat zu Berlin, Biochemie der Pflanze, Invaliden Strasse 43, 10115 Berlin, Germany*
[b] *Universität Regensburg, Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universitätstrasse 31, 8400 Regensburg, Germany*
[c] *Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA*

Received 20 January 1995; revised 20 March 1995; accepted 5 April 1995

Abstract

The genes encoding subunits A and B of V-ATPase in *Cyanidium caldarium* were cloned and sequenced. While the gene encoding subunit A is not interrupted by introns, the gene encoding subunit B contains seven introns ranging from 36 to 60 nucleotides.

Keywords: ATPase, V-; Gene; Intron; Bioenergetics; (*C. caldarium*)

1. Introduction

Numerous organelles of the vacuolar system of eukaryotic cells are energized by V-ATPases and each organelle has a specific requirement for its internal pH and membrane potential [1–5]. Thus endosomes operate with smaller ΔpH than the vacuoles or lysosomes, and therefore their V-ATPase has to be regulated in order to obtain the required protonmotive force for each of the organelles. These special requirements may be fulfilled by secondary transport systems such as chloride carriers and regulation of the V-ATPase activity. The internal pH of organelles of the vacuolar system is also variable and tightly regulated. While yeast vacuoles maintain an internal pH of about 5.5 units, it is assumed that the vacuoles of lemon fruit may have a pH as low as 2 units [6]. Similarly, some brown and red alga maintain very low pH in their vacuolar system. Further evidence that the V-ATPase is regulated in plants is provided by the fact that the vacuolar pH can vary in different tissues of the same plant and in the same cell during the course of development or in response to changing environmental conditions. Crassulacean Acid Metabolism (CAM) plants are the classic example of vacuolar pH regulation. The pH of their leaf vacuoles fluctuate from pH 3 at night to pH 6 in the day [7]. During the maturation of citrus lemon fruit the vacuolar pH of the juice sac cells declines from 5.5 to about 2. This may suggest that in the mature lemon fruit V-ATPase functions at a thermodynamic equilibrium. In most cases V-ATPase operates below thermodynamic equilibrium and it was proposed that a proton slip is one of the main factors in maintaining smaller ΔpH in different organelles of the vacuolar system [8]. We elected to utilize the thermophylic and acidophylic unicellular red alga *Cyanidium caldarium* to investigate the underlying mechanism of maintaining relatively low internal pH in their vacuole. The red alga *Cyanidium caldarium* grows in a medium with a pH of 2.5 to 2.7. We assume that the pH of its vacuoles may be lower than the external medium. If this is the case, cloning and sequencing of V-ATPase subunits may reveal the clue for regulating the internal pH of organelles of the vacuolar system. Here we describe the cloning of two genes encoding V-ATPase subunits, subunit A and subunit B from *Cyanidium caldarium*.

Cyanidium caldarium was routinely grown in a 12 l glass flask under constant illumination and aeration at a temperature of about 42° C. The composition of the medium was: $(NH_4)_2SO_4$, 1 g/l: K_2HPO_4, 20 mg/l; $MgSO_4$ 7 H_2O, 20 mg/l; micronutrient solution for BG-11 medium [23], 1 ml/l; yeast extract, 0.1% (w/v); glucose 0.2% (w/v); pH 2.7. Cells were harvested by centrifugation, washed twice with STN-buffer (330 mM sorbitol, 30 mM Tricine, 3 mM EDTA (pH 7.5)), redissolved in STN-buffer and stored frozen at −20° C.

DNA of *Cyanidium caldarium* was prepared according to a published method of Phenol/SDS that was developed

* Corresponding author. Fax: +1 201 2355848.

SSDI 0005-2728(95)00060-7

```
   1  GGATCCTATCCATACTGTCCAGTTTTCCAATCACACTGGATATTTAGGTGGCTTTCGTGG   60
  61  AAAGAGACTGAGCAGTGAAGAGTTTGATCAACTCATTGCTGGTTTAGAGGCCAACTGTTT  120
 121  GTTGCAACAAGTGTAAGTATCATTGATGGAAGTAGGTCAGCGGTGTTTTGACGACGCATA  180
 181  GGGATTATGTCTTGATGGGATATATAGGTGACCATGATTTGCTACTTCATATATTGGAAG  240
 241  CCGTCAAAAAACTCAAGAAACCCAATCTTGTCTTTGTATGTGATCCCGTAATGGTAGGTA  300
 301  CTGCTATGGATGGAATAATATATAGTCCTTAGTTGGGTGTGTCATAGGGCGATAATGGGA  360
 361  AATTGTATGTTCCTCCAAGTCTCGTTCCTATTTATCGAGACCAAGTAATTATGCTTGCCG  420
 421  ATATTATAACACCGAATCAGTTCGAGTTGTCCATCCTATCCGAGAAAGCTGTGGGTTCCG  480
 481  TTACAGAGGCATTCCAAGCTTGTAGATATTTACATGAATATCGAAAAGTACAGAATATTG  540
 541  TAGTTACTAGTGGAGAATACGAAGATATGGATTCTTTTGTGATACTGATAAGTAGTGACT  600
 601  TTGGTAACCAAAAATATATTCAGACAGTAGAGAAGATACAAGGTAGTTTCACGGGAGCTG  660
 661  GTGACTTGAGTAGTGCTCTTATTCTTGGCTGGTATGTTATTTTGAAAGGTGATATTGTAG  720
 721  CTGCTTGTGAAAGAGCAATGGCAAGTGTTCACCGTACTTTGGTACATACGGCAGCCGTAC  780
 781  AATCGCCGCCAACTCGGTGGTGGGAATTGGAATTGATTGGCAGTCAACTTTGGTTGAGGA  840
 841  ATCCTCCGTTAGGCATCGTGAAGACTCGTGCGATTACACTACAAGATGACAACAGTTCGA  900
                                                   M  T  T  V  R
 901  GTCAACGGAATGAAAAACGGAATAATTAAAAAAGTATCCGGTCCTGTCGTTTCCGCAGAA  960
      V  N  G  M  K  N  G  I  I  K  K  V  S  G  P  V  V  S  A  E
 961  AATATGGACGGTGCTGCAATGTATGAACTCGTTCGTGTAGGAAACGAACAACTGGTGGGT 1020
      N  M  D  G  A  A  M  Y  E  L  V  R  V  G  N  E  Q  L  V  G
1021  GAAATCATCCGTTTGGAGGGTTCCGTTGCGACAATTCAAGTGTACGAAGAAACTTCAGGA 1080
      E  I  I  R  L  E  G  S  V  A  T  I  Q  V  Y  E  E  T  S  G
1081  TTAACTATAGGTGACCCAGTATTGTGTACTGGATCGCCACTATCCGTAGAATTGGGACCA 1140
      L  T  I  G  D  P  V  L  C  T  G  S  P  L  S  V  E  L  G  P
1141  GGCTTGATGGGAAATATTTTCGACGGCATACAAAGACCACTCGAGAAAATTGCGGAAAGA 1200
      G  L  M  G  N  I  F  D  G  I  Q  R  P  L  E  K  I  A  E  R
1201  AGCAACTCTGTGTTTATACCAAGAGGAGTAAACGTTCCGGCTTTAGATAGAAAGAAAGTA 1260
      S  N  S  V  F  I  P  R  G  V  N  V  P  A  L  D  R  K  K  V
1261  TGGGAGTTTAGACCTGCAGACAACCTAAAAGTAGGAGACCCGATAACTGCTGGAGATATA 1320
      W  E  F  R  P  A  D  N  L  K  V  G  D  P  I  T  A  G  D  I
1321  TACGGCATCGTTCCTGAAACACCTCTAATAGACCACAAGATTATGTTACCGCCAAACCAA 1380
      Y  G  I  V  P  E  T  P  L  I  D  H  K  I  M  L  P  P  N  Q
1381  ATGGGAAAGATAGTGTTTCTAGCGCCACCTGGAGACTATACTTTGGAAGATACTGTTTTG 1440
      M  G  K  I  V  F  L  A  P  P  G  D  Y  T  L  E  D  T  V  L
1441  GAAATAGACTTCAATGGACAAAAGAAAAAGTTCTCCATGGTACATCAGTGGCCAGTAAGA 1500
      E  I  D  F  N  G  Q  K  K  K  F  S  M  V  H  Q  W  P  V  R
1501  CTTCCTAGACCAGTGACAGAAAAACTTCGGGCAGATAAACCACTTTTAACCGGTCAAAGG 1560
      L  P  R  P  V  T  E  K  L  R  A  D  K  P  L  L  T  G  Q  R
1561  GTATTGGACGCTCTCTTCCCTTCGGTACAAGGAGGAACTTGTGCTATACCAGGTGCTTTT 1620
      V  L  D  A  L  F  P  S  V  Q  G  G  T  C  A  I  P  G  A  F
1621  GGTTGTGGAAAGACAGTTATCTCACAAGCACTGAGTAAATTTTCAAACTCAGATGGAATA 1680
      G  C  G  K  T  V  I  S  Q  A  L  S  K  F  S  N  S  D  G  I
1681  GTTTATGTCGGTTGTGGGGAAAGAGGCAATGAAATGGCAGAAGTATTGAAGGACTTTCCG 1740
      V  Y  V  G  C  G  E  R  G  N  E  M  A  E  V  L  K  D  F  P
1741  GAACTAACCATGACAGTGGGAGATAGAGAAGAAAGTATCATGAAAAGAACTCTTCTGGTA 1800
      E  L  T  M  T  V  G  D  R  E  E  S  I  M  K  R  T  L  L  V
1801  GCAAATACTTCCAATATGCCTGTGGCTGCTAGAGAAGCTTCCATTTATACCGGAATTACT 1860
      A  N  T  S  N  M  P  V  A  A  R  E  A  S  I  Y  T  G  I  T
1861  GTCTCGGAATATTACAGAGATATGGGTTTAAATATAAGTATGATGGCCGACTCTACTTCC 1920
      V  S  E  Y  Y  R  D  M  G  L  N  I  S  M  M  A  D  S  T  S
1921  AGATGGGCAGAAGCCTTGAGAGAAATAAGTGGAAGACTGGCGGAAATGCCGGCAGATAGT 1980
      R  W  A  E  A  L  R  E  I  S  G  R  L  A  E  M  P  A  D  S
1981  GGTTATCCTGCATATTTGGCAGCTAGATTGGCAAGTTTTTATGAGAGAGCTGGTAAGGTA 2040
      G  Y  P  A  Y  L  A  A  R  L  A  S  F  Y  E  R  A  G  K  V
2041  TCTTGTTTAGGCTCCCCTAATAGACAAGGTTCTATTACTATTGTGGGAGCTGTATCACCA 2100
      S  C  L  G  S  P  N  R  Q  G  S  I  T  I  V  G  A  V  S  P
2101  CCAGGTGGTGACTTTTCAGATCCTGTTACGTCAGCCACCCTTGGAATCGTTCAAGTCTTT 2160
      P  G  G  D  F  S  D  P  V  T  S  A  T  L  G  I  V  Q  V  F
2161  TGGGGTTTGGATAAGAAACTGGCTCAACGAAAGCATTTTCCTTCGGTCAATTGGCTCATC 2220
      W  G  L  D  K  K  L  A  Q  R  K  H  F  P  S  V  N  W  L  I
2221  TCCTATTCCAAGTACATGAAGGCATTGGAGCCGTATTACGAAGAACGGTTCCCGGAATTT 2280
      S  Y  S  K  Y  M  K  A  L  E  P  Y  Y  E  E  R  F  P  E  F
2281  CTAAACTATCAACAAAAGGCTAGAGAAATTCTTCAAACAGAGGATGATCTAATGGAAATA 2340
      L  N  Y  Q  Q  K  A  R  E  I  L  Q  T  E  D  D  L  M  E  I
2341  GTACAACTAGTAGGGAAGGACTCGCTGGCAGAAAACGATAAGATTACGCTGGAAGTTGCA 2400
      V  Q  L  V  G  K  D  S  L  A  E  N  D  K  I  T  L  E  V  A
2401  AAAATGATACGTGAAGACTTTCTTGCGCAAAATTCGTTTACTGAATATGACCGATTCTGT 2460
      K  M  I  R  E  D  F  L  A  Q  N  S  F  T  E  Y  D  R  F  C
2461  CCATTTTACAAGAGCGTTCTCATGTTGCGCAATATGATTCATTTTTATGAGTTGGCAAAT 2520
      P  F  Y  K  S  V  L  M  L  R  N  M  I  H  F  Y  E  L  A  N
2521  AAGGCAGTTGAAGGATCCGGTGAGCAACATCTAACATTGGCGCAGATAAAGGAACAAATG 2580
      K  A  V  E  G  S  G  E  Q  H  L  T  L  A  Q  I  K  E  Q  M
2581  GGTGAAACCATCTACAAGATATCTGGTATGAAGTTTTTGGATCCTGCACAAGGTTGGTCT 2640
      G  E  T  I  Y  K  I  S  G  M  K  F  L  D  P  A  Q  G  W  S
2641  CTTTTTTGAACATTGCACTGGGTTTATATTACTGGGTTGTTAGGTGAAGATGCTTTGAGA 2700
      L  F  *
2701  AGTAAGCTCGATGCACTGTACTCGCAGATAACAGACGGCTTTTACAAAATGGAGAATAGT 2760
2761  CTATGACATTAAGGAAACAAGTATATAAAGATGTTCGCAGTGAATATTTAGTTGCGTTGT 2820
2821  TTGTCGAACAGTCTTTGGTATACTTCACAAAAAAGCCTTTATCGTAATACAAAACGGCAA 2880
2881  CACTCAAAACGGGTAGAAGCACAGAAGATATCTCAACGAATTGCGCCAAATCATCTCCGT 2940
2941  CCACTTTTTTCAAAATTTTTTTCGTCTTTTTTTTTTGGCGCCAACTTTTGGGATTTTGGC 3000
3001  GCCTTCACTTTTCCGTACAAAATTTTTCTATTCTTGTAGGAAAAGGATGACAGAGTGGGA 3060
3061  TGCTCAACCAGTGTATTACGGTGCTCCAACCTTTCAGAATAATGAAAGGAATATAAATGA 3120
3121  ACTGTCAAGATTCGCAGCAAAAGAAAAATTTAGAGAGTTCTTCTTGCACTACACTTCCAG 3180
3181  TGAATCATGTTTCAAGTATAGAGAGAAGCTT  3211
```

Fig. 1. Nucleotide and deduced amino acid sequences of the gene encoding subunit A of V-ATPase from *Cyanidium caldarium*. GenBank accession number U17100.

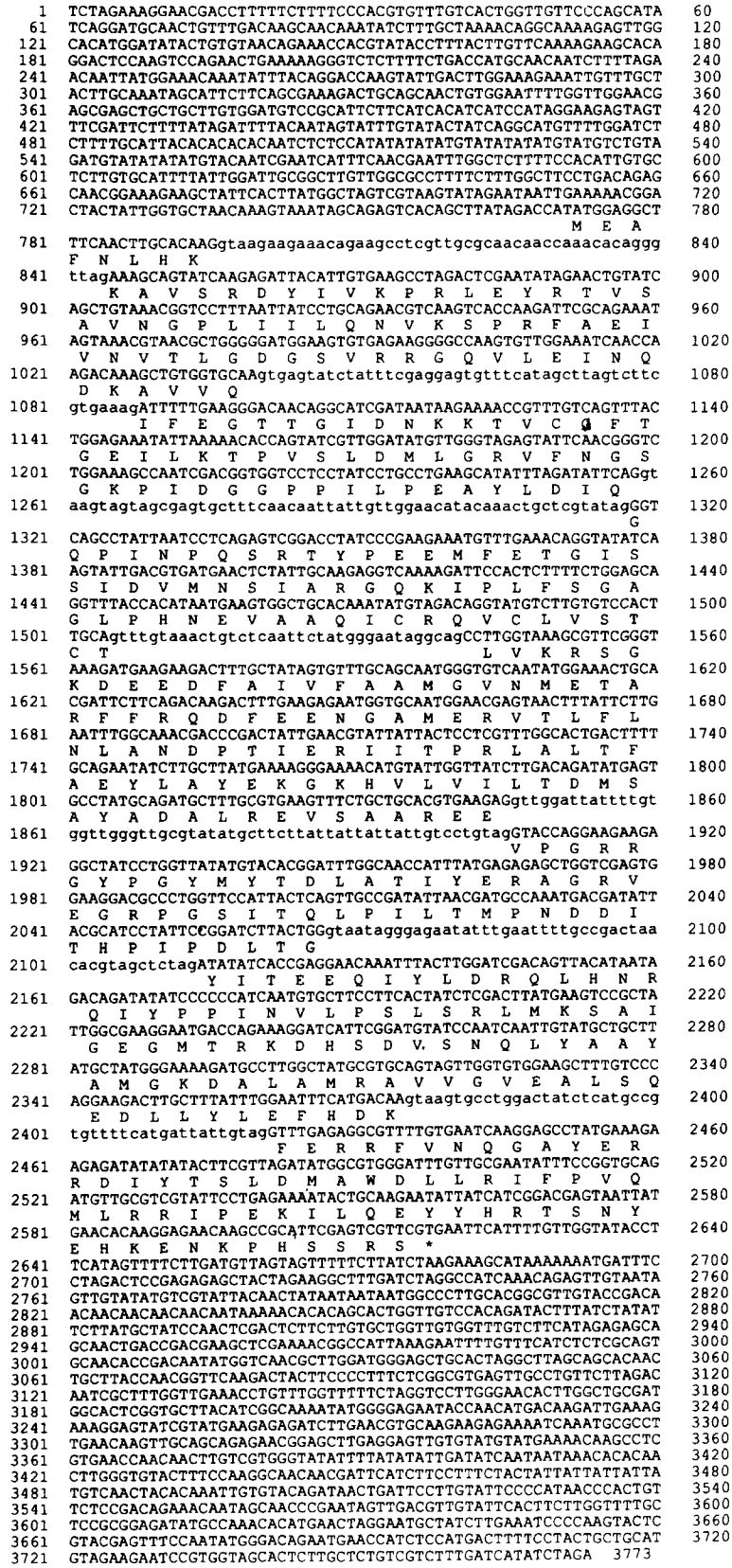

Fig. 2. Nucleotide and deduced amino acid sequences of the gene encoding subunit B of V-ATPase from *Cyanidium caldarium*. GenBank accession number U17101.

for preparation of plant DNA [9]. All the DNA libraries were constructed in the same way: *Cyanidium caldarium* DNA was digested completely with restriction enzyme, fragments of appropriate size were isolated and cloned into the respective restriction site of plasmid pBluescript II SK +. The resulting constructs were used for transformation of competent *E. coli* strain DH5α, ampicillin resistant cells were recovered. Screening of the libraries was carried out with ^{32}P-labeled probes by repeated colony screening and subsequently by dot blot analysis [10]. Labeling of the probes was performed by Random priming with Klenow-Polymerase.

For sequencing the cloned fragments, overlapping deletions were generated by the method of Henikoff by unidirectional digestion with exonuclease III [11]. The resulting sets of truncated DNA and the original fragments were sequenced with T_3 and T_7 promoter primers. Second DNA strand was sequenced using internal primers.

Screening for the gene of subunit A

A DNA library was constructed from BamHI-DNA fragments of 1.5 to 10 kb clone into *Bam*HI site in pBluescript SK +. The resulting library consisted of about 20 000 independent colonies. Screening for the gene encoding subunit A was performed by using a 0.8 kb cDNA fragment from bovine adrenal medulla together with a 0.65 kb fragment of the corresponding yeast gene. About 70 000 colonies of the *Cyanidium* library were screened and the positive colonies were analyzed by dot blot. Four clones giving strong signals with both probes were obtained. All of them contained the same insert of 2538 bp (nucleotides 1 to 2538 of the sequence). Sequencing showed that the DNA fragment is missing the part of the gene encoding the C terminal part of the protein. A partial DNA library of 1.0 to 1.35 kb *Hin*dIII fragments of *Cyanidium* DNA was prepared and screened with a 0.19 kb *Spe*I/*Bam*HI fragment of the *Cyanidium* gene (nucleotides 2346 to 2538 of the sequence) as a probe. Three positive clones were obtained, all contained the same insert of 1371 nucleotides (nucleotides 1840 to 3211 of the sequence). Sequence analysis of the *Bam*HI and *Hin*dIII DNA fragments showed that both overlapped with about 700 bp. The alignment of the sequences resulted in the complete sequence of the *Cyanidium* gene of subunit A.

1.2. Screening for the gene of subunit B

For isolating the gene encoding subunit B, a 1.1 kb bovine cDNA together with a 1.4 kb fragment of the corresponding yeast gene were used as probes [20]. About 120 000 colonies of the above *Cyanidium* library were screened with the above labeled DNA fragments and positive colonies were analyzed by dot blot hybridization. Four clones which gave strong signals with both probes were sequenced and showed high homology to genes encoding subunit B of V-ATPase from other sources. After *Xba*I digestion, two fragments of about 1.7 and 2.1 kb were identified by Southern blots analysis. Both fragments were cloned into pBluescript II SK + and sequenced. The analysis of the sequences showed that each of the 2 *Xba*I fragments contained a part of the gene and when aligned gave the complete sequence of the gene encoding subunit B.

Fig. 1 shows the nucleotide and deduced amino acid sequences of the Cyanidium gene encoding subunit A of V-ATPase. The deduced amino acid sequence contains 587 amino acids and it is highly homologous to other amino acid sequences of subunit A of V-ATPase from other sources. A search in the GenBank revealed that the *Cyanidium* subunit A is 73% identical to the corresponding subunit from bovine [13] and about 70% identical to the corresponding subunits from the insect *Manduca sexta*, the fungi *Neurospora crassa* and *Saccharomyces cerevisiae* or the plant carrot [14–16]. It also exhibited over 50% identity with the corresponding subunit A of the enzymes from *Archaebacteria* [17,18]. Its high identity with subunits from mammalian sources vs. those of fungal and plant is somewhat surprising. The open reading frame is uninterrupted with exons and it has no unusual features. In contrast to subunit A, the gene encoding subunit B is interrupted by 7 introns (Fig. 2). The size of the intron ranges from 36 nucleotides up to 60 nucleotides. After computer splicing of the introns the reading frame contains 500 amino acids. The deduced protein is highly homologous to subunit B from other sources. It exhibits nearly 80% identity with the corresponding V-ATPase subunits from bovine, plants and the insect *Manduca sexta* [19,20]. The corresponding subunit of *Saccharomyces cerevisiae* and *Neurospora crassa* are only about 72% identical to the *Cyanidium* subunit [12,21]. As subunit A, subunit B also has over 50% identity with the corresponding subunit from archaebacteria [18,22]. Table 1 depicts the percentage identity of the amino acid sequences of subunits A and B

Table 1
Sequence homology of the *Cyanidium* subunits A and B with corresponding subunits from various sources

Source	Subunit	% Identity	Accession number	Ref.
Bovine	A	73	X58386	[24]
	B	77	M83131	[19]
Human	A	73	L09235	[25]
	B	76	X62949	[26]
Manduca sexta	A	70	S94847	[27]
	B	77	S43760	[28]
Carrot	A	73	J03769	[16]
A. thaliana	B	80	J04185	[20]
S. cerevisiae	A	70	J05409	[14]
	B	72	J04450	[12]
Entamoeba histolytica	A	70	U04849	[28]
Plasmodium	A	63	L08200	[29]
falciparum	B	69	U03915	[30]
Trypanosoma	A	68	Z25814	–
congolense	B	71	Z25815	–

of *Cyanidium* V-ATPase with corresponding subunits from other sources. The percentage identities suggest closer relation to mammalian subunits than to the fungal ones.

The gene structure encoding subunit A and subunit B is quite interesting. While the gene encoding subunit A has no intervening sequences, the one encoding subunit B contains several of them. Being unaware of other nuclear genes that were cloned and sequenced from *Cyanidium caldarium*, we cannot know whether this is a general pattern of this organism or if it is a unique situation for the V-ATPase subunit. An attempt to identify the source of the two genes evolutionary tracing did not give significant results (Lill and Nelson, unpublished). Further studies on the genes encoding other subunits of V-ATPase from *Cyanidium caldarium* may shed light on the control mechanisms of internal pH in the vacuolar system.

References

[1] Mellman, I., Fuchs, R. and Helenius, A. (1986) Annu. Rev. Biochem. 55, 663–700.
[2] Nelson, N. (1989) J. Bioenerg. Biomembr. 21, 553–571.
[3] Nelson, N. (1992) Biochim. Biophys. Acta 1100, 109–124.
[4] Taiz, L. (1992) J. Exp. Biol. 172, 113–122.
[5] Forgac, M. (1989) Physiol. Rev. 69, 765–796.
[6] Sze, H. (1985) Annu. Rev. Plant Physiol. 36, 175–208.
[7] Luttge, U. (1987) New Phytol. 106, 593–629.
[8] Moriyama, Y. and Nelson, N. (1988) in The Ion Pumps, Structure, Function and Regulation (Stein, W.D., ed.), pp. 387–394, Alan R. Liss, New York.
[9] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1987) Current Protocols in Molecular Biology, Vols. I and II, John Wiley & Sons, New York.
[10] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Harbor Laboratory, New York.
[11] Henikoff, S. (1984) Gene 28, 351–359.
[12] Nelson, H., Mandiyan, S. and Nelson, N. (1989) J. Biol. Chem. 264, 1775–1778.
[13] Puopolo, K., Kumamoto, C., Adachi, I. and Forgac, M. (1991) J. Biol. Chem. 266, 24564–24572.
[14] Hirata, R., Ohsumi, Y., Nakano, A., Kawasaki, H., Suzuki, K. and Anraku, Y. (1990) J. Biol. Chem. 265, 6726–6733.
[15] Bowman, E.J., Tenney, K. and Bowman, B.J. (1988) J. Biol. Chem. 263, 13994–14001.
[16] Zimniak, L., Dittrich, P., Gogarten, J.P., Kibak, H. and Taiz, L. (1988) J. Biol. Chem. 263, 9102–9112.
[17] Denda, K., Konishi, J., Oshima, T., Date, T. and Yoshida, M. (1988) J. Biol. Chem. 263, 6012–6015.
[18] Inatomi, K.I., Eya, S., Maeda, M. and Futai, M. (1989) J. Biol. Chem. 264, 10954–10959.
[19] Puopolo, K., Kumamoto, C., Adachi, I., Magner, R. and Forgac, M. (1992) J. Biol. Chem. 267, 3696–3706.
[20] Manolson, M.F., Ouellette, B.F.F., Filion, M. and Poole, R.J. (1988) J. Biol. Chem. 263, 17987–17994.
[21] Bowman, B.J., Allen, R., Wechser, M.A. and Bowman, E.J. (1988) J. Biol. Chem. 263, 14002–14007.
[22] Denda, K., Konishi, J., Oshima, T., Date, T. and Yoshida, M. (1988) J. Biol. Chem. 263, 17251–17254.
[23] Schlosser, U.G. (1994) Botan. Acta 107, 113–186.
[24] Pan, Y.X., Xu, J., Strasser, J.E., Howell, M. and Dean, G.E. (1991) FEBS Lett. 293, 89–92.
[25] Van Hille, B.J.M., Richener, H., Evans, D.B., Green, J.R. and Bilbe, G. (1993) J. Biol. Chem. 268, 7075–7080.
[26] Bernasconi, P., Rausch, T., Struve, I., Morgan, L. and Taiz, L. (1990) J. Biol. Chem. 265, 17428–17431.
[27] Graf, R., Novak, F.J., Harvey, W.R. and Wieczorek, H. (1992) FEBS Lett. 300, 119–122.
[28] Yi, Y. and Samuelson, J. (1994) Mol. Biochem. Parasitol. 66, 165–169.
[29] Karcz, S.R., Herrmann, V.R. and Cowman, A.F. (1993) Mol. Biochem. Parasitol. 58, 333–344.
[30] Karcz, S.R., Herrmann, V.R. and Cowman, A.F. (1994) Mol. Biochem. Parasitol. 65, 123–133.